streptococci as discussed in the present application on pages 5 (line 18) to 7 (line 2). In view of this disclosure, applicants request that this rejection be withdrawn.

Claims 31-35 and 48-50 were rejected under 35 USC §101. Claims 31-35 and 48-50 have been canceled rendering this rejection moot.

Claims 1-19, 21, 26-42 and 48-52 were rejected under 35 USC §112, second paragraph as indefinite. Claims 1-19, 21, 26-42 and 48-52 have been canceled and new claims added to the application which do not include the language found indefinite. Applicants point out that the term "presumptive medium" is explained on page 19, lines 21-38 of the present application. In view of this disclosure, applicants contend that this term is not indefinite. In view of the cancellation of claims 1-19, 21, 26-42 and 48-52, applicants request that this rejection be withdrawn.

Claims 48, 50, 51 and 52 were rejected under 35 USC 102(a) as anticipated by Pia. A certified translation of the priority document is being prepared and will be filed shortly. Applicants request that this rejection be held in abeyance until the translation is filed as the priority document is from May 22, 1998 and thus Pima is not available as prior art against the present application.

Claims 51 and 52 were rejected under 35 USC §102(e) as anticipated by Stover and also Meyers. The sequences disclosed by Stover and Meyers are each more than 10,000 bases in length. Claims 51 and 52 are directed to significantly smaller oligonucleotides which can be used as amplification primers or hybridization probes. Claims 51 and 52 have been canceled and new claims added to the application which clarify the present invention.

Claims 36-42 were rejected under 35 USC §103(a) as unpatentable over Morotomi in view of the 1988 Strategene catalog. Neither of these references discloses or suggests a reagent kit for determining macrolide antibiotic resistance in microorganisms by in-situ hybridization. Therefore, in view of the cancellation of claims 36-42 and the addition of new claims to the application, applicants request that this rejection be withdrawn.

Claims 36-41 were rejected under 35 USC §103(a) as unpatentable over Pina in view of the 1988 Strategene catalog. As discussed above, Pina is after the priority date of the present application. A certified translation of the priority document is being prepared and will be submitted shortly. Applicants ask that this rejection be held in abeyance until the certified translation is submitted.

Claim 42 was rejected under 35 USC §103(a) as unpatentable over Pina in view of the 1988 Strategene catalog further in view of Morotomi. Neither Morotomi or the 1988 Strategene catalog suggest or disclose a reagent kit for determining macrolide antibiotic resistance in microorganisms by in-situ hybridization. In addition, as discussed above, Pina is after the priority date of the present application. A certified translation of the priority document is being prepared and will be submitted shortly. Applicants ask that this rejection be held in abeyance until the certified translation is submitted.

Claims 1-19, 21, 26-35 and 49 were rejected under 35 USC 103(a) as unpatentable over Pina in view of Amann. As discussed above, a certified translation of the priority document is being prepared and will be submitted shortly showing that Pina is not available as prior art against the present application. Amann discloses the typing of microorganisms using in situ hybridization, i.e. the determination of specific types of microorganisms in a sample. Amann does not suggest using his procedure for the determination of antibiotic resistance as in the present invention. When determining antibiotic resistance, the choice of hybridization probe is restricted to the region containing the causal mutation. In contrast to this, when typing microorganisms, for example in the differentiation of sub-types as described in Amann, a plurality of different regions in the genome of the relevant microorganism can be used as the target sequence for the probes. This restriction to the specific regions containing the causal mutation would have deterred one skilled in the art from applying the process of the invention, since not every sequence in the target region is suitable. For example, DNA sequences can be in a region which is not accessible for hybridization (e.g. regions occupied by histones or which are supercoiled), or RNA sequences can include ribosomes. In particular, the

resistance can often be ascribed to a point mutation in a gene or a portion of the RNA. One skilled in the art would not expect hybridization in situ to be suitable as explicit and reliable evidence of point mutations in macrolide antibiotic resistance as shown in the present invention. These concerns are confirmed by Amann (page 765, right column, paragraph 3), where he indicates that while it was possible to distinguish between strains that differed by a single mismatch, the ability to discriminate complementary from single mismatch hybrids varied markedly between different probe and target sequences. In other words, differentiation between target and detection sequences with individual inappropriate base pairings is possible in some cases but the possibility of discrimination between different sequences varies considerably. This is further confirmed by Amann where the differentiation between individual fibrobacter species through in situ hybridization with fluorescence labeled oligonucleotides is described (page 767, right column). Here a weak detection reaction was observed with the detection probe for fibrobacter isolation in both of the subtypes JG1 and LH1. A point mutation existing in the subtypes could only be detected and differentiated from the initial sequence by sequencing. Amann's discussion would thus lead one away from the use of in situ hybridization as evidence of macrolide antibiotic resistance since resistance is often due to a point mutation in a gene or a portion of the RNA and one would expect sequencing to be necessary to accurately detect the point mutations.

Applicant's also point out that in Amann the detection reaction is carried out with samples containing cultivated and isolated microorganisms, while in the process of the invention an in situ hybridization is carried out in complex biological samples. A specific and reliable determination could not have been predicted due to the autofluorescence of the microorganism sample matrices, e.g. of tissue. In view of the above discussion, and after the submission of the certified translation of the priority document, applicants request that this rejection be withdrawn.

Claims 1-19, 21, 26-35 and 48-52 were rejected under 35 USC §103(a) as unpatentable over Versalovic in view of Amann. Versalovic teaches a method for detecting mutations in 23S

rRNA associated with clarithromycin resistance in Helicobacter pylori by sequencing the nucleic acids of H. pylori. Versalovic does not suggest that antibiotic resistance in microorganisms can be detected by in situ hybridization as in the present invention. Amann does not cure this deficiency because as discussed above, Amann teaches away from the use of in situ hybridization for detecting point mutations (such as those underlying macrolide antibiotic resistance) and does not suggest that discrimination from wild type strains is possible. Contrary to statements made in the Office Action, Amann does not teach the effective detection of a single point mutation in a bacterial genome. Amann states on page 765 that the ability to discriminate complementary from single mismatch hybrids varied markedly between different probe and target sequences and that in general, single mismatch discrimination must be empirically established. In addition, page 767 indicates that rRNA sequencing was required to verify the mismatch. In view of the above discussion, applicants request that this rejection be withdrawn.

Claims 36-41 were rejected under 35 USC §103(a) as unpatentable over Versalovic in view of Amann and the Strategene catalog. As discussed above, neither Amann or Versalovic suggest or disclose that macrolide antibiotic resistance can be determined using in situ hybridization. Strategene does not sure this deficiency as Strategene discloses only reagent kits without suggesting reagents which would be suitable for detecting macrolide antibiotic resistance which is often due to point mutations. In view of this, applicants request that this rejection be withdrawn.

Claim 42 was rejected under 35 USC §103(a) as unpatentable over Versalovic in view of Amann, Strategene and Morotomi. Morotomi is cited for the disclosure of a urease inhibitor but does not cure the above discussed deficiencies in Versalovic, Amann and Strategene. Specifically, none of these references individually or in combination suggest that in situ hybridization can be used to determine macrolide antibiotic resistance which is often due to a single point mutation. Therefore, the combination of these references does not suggest an in situ hybridization reagent kit containing a hybridization probe which is specific for a nucleic

acid sequence which is associated with antibiotic resistance. In view of the above discussion, applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 53-104 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

<table>
<tr><td colspan="6">RESPECTFULLY SUBMITTED,</td></tr>
<tr><td>NAME AND REG. NUMBER</td><td colspan="5">Monica Chin Kitts, Registration No.: 36,105</td></tr>
<tr><td>SIGNATURE</td><td colspan="2">  </td><td>DATE</td><td colspan="2">2/6/03</td></tr>
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</table>

Marked up Tables

Name	Probe Sequence (5' - 3')	Target region* rRNA	Binding Specificity
Hpy1-16S-753	GCTTTCGCGCAATCAGCG SEQ ID No:5	753-770 (16S)	H. pylori
120 b	AGGCACATGATCTATGCG SEQ ID No:6	120-137 (16S)	H. pylori
Hpyl-16S-585	CACACCTGACTGACTATCCCG SEQ ID No:7	585-605 (16S)	H. pylori H. nemestrinae
Hpyl-16S-219	GGACATAGGCTGATCTCTTAGC SEQ ID No:8	219-240 (16S)	H. pylori
Hh1	CCCACACTCCAGAAG (G/A) ATAG SEQ ID No:9	644-663 (16S)	H. heilmannii
Hh2	CCCACACTCTAGGGTT (G/T) GCAG SEQ ID No:10	644-664 (16S)	H. heilmannii
Hh3	CCCACACTCTAGAAAGATAG SEQ ID No:11	644-663 (16S)	H. heilmannii
Hh4	CACATCTGACTTGCCACCCCG SEQ ID No:12	585-605 (16S)	H. heilmannii
ClaR1	CGGGGTCTTCCCGTCTT SEQ ID No:1	2051-2067 (23S)	A2058G (Cla^R)
ClaR2	CGGGGTCTCTCCGTCTT SEQ ID No:2	2051-2067 (23S)	A2059G (Cla^R)
ClaR3	CGGGGTCTTGCCGTCTT SEQ ID No:3	2051-2067 (23S)	A2058C (Cla^R)
ClaWT	CGGGGTCTTTCCGTCTT SEQ ID No:4	2051-2067 (23S)	Wild type (Cla^R)

Marked up Tables

Page 41, please delete table 7 and insert the following new table 7:

Table 7:
Comparison of the 23S rRNA sequences of various bacterial species within the clarithromycin resistance region

Probe sequence		5'-CGGGGTCTTTCCGTCTT-3 SEQ ID No:4
rRNA sequence	mis	5'-AAGACGGAAAGACCCCG-3 SEQ ID No:13
Helicobacter pylori claWT	0	ACCCGCGGC-==========-UGGACCUUU
Helicobacter pylori claR1	1	ACCCGCGGC-======G=========-UGGACCUUU
Helicobacter pylori claR2	1	ACCCGCGGC-=======G========-UGGACCUUU
Helicobacter pylori claR3	1	ACCCGCGGC-======C========UGGACCUUU
Campylobacter jejuni	0	ACCCGCGGC-==========-UGGACCUUU
Campylobacter coli	0	ACCCGCGGC-==========-UGGACCUUU
Wolinella succinogenes	0	ACCCGCGGC-==========-UGGACCUUU
Nannocystis exedens	0	ACCCGCGGC-==========-UGAACCUUU
Escherichia coli	0	ACCCGCGGC-==========-UGAACCUUU
Salmonella typhi	0	ACCCGCGGC-==========-UGAACCUUU
Enterobacter cloacae	0	ACCCGCGGC-==========-UGAACCUUU
Citrobacter freundii	0	ACCCGCGGC-==========-UGAACCUUU
Klebsiella pneumoniae	0	ACCCGCGGC-==========-UGAACCUUU
Yersinia pestis	0	ACCCGCGGC-==========-UGAACCUUU
Plesiomonas shigelloides	0	ACCCGCGGC-==========-UGAACCUUU
Haemophilus influenzae	1	ACCCGCGGC-U=========-UGAACCUUU
Vibrio vulnificus	1	ACCCGCGGC-U=========-UGAACCUUU
Aeromonas hydrophila	1	ACCCGCGGC-U=========-UGAACCUUU
Pseudomonas aeruginosa	1	ACCCGCGGC-U=========-UGAACCUUU
Acinetobacter calcoaceticus	1	ACCCGCGGC-U=========-UGAACCUUU
Neisseria meningitidis	1	ACCCGCUGC-U=========-UGAACCUUU
Bordetella pertussis	2	ACCCGCGGC-U=========A-UGAACCUUU
Bartonella bacilliformis	1	UCCUGCGGU-U=========-UGCACCUUU
Rickettsia rickettsii	1	UCCCGCGGU-C=========-UGAACCUUU
Borrelia burgdorferi	1	ACUUGUGGU-U=========-UGAACCUUU
Leptospirillum ferrugineum	2	CCCCGCGGC-U=========U=-UGCACCUUU
Listeria monocytogenes	1	ACCCGCGAC-=G========-UGGAGCUUU
Staphylococcus aureus	1	ACCCGCGAC-=G=========-UGGAGCUUU
Bacillus anthracis	1	ACCCGCGAC-=G=========N=-UGGAGCUUU
Mycoplasma hyopneumoniae	1	ACCCGCAUC-=====A=========-UGGAGCUUU
Mycoplasma pneumoniae	2	AGGCGCAAC-GG=========-UGAAGCUUU
Streptococcus parauberis	2	ACCCGCGAC-=G=========A-UGGAGCUUU
Lactococcus lactis	2	ACCCGCGAC-=G=========A-UGGAGCUUU
Enterococcus faecalis	2	ACCCGCGAC-=G=========A-UGGAGCUUU
Clostridium botulinum	2	ACCCGCGAU-UG=========-UAGAGCUUU
Streptomyces griseus	1	UCGCGCAGC-=G=========-GGACCUUUA
Micrococcus luteus	1	ACGCGCAGA-=G=========-UGACCUUUA
Corynebacterium glutamicum	1	ACGCGCGGC-=G=========-GGACCUUCA
Gardnerella vaginalis	1	AAGCGCAGA-=G=========-GGACCUUUA
Mycobacterium leprae	2	ACGUGCGGC-=G====A=========-GGACCUUCA
Bifidobacterium bifidum	2	AAGCGCAGA-=G====A=========-GGACCUUUA
Chlamydia trachomatis	2	ACCCGCGAA-=G====A=========-UGAACCUUU
Chlamydia pneumoniae	2	CCCCGCAAA-=G====A=========-UGAACCUUU
Bacteroides fragilis	3	ACCCGCGAU-GG====A=========-UGAACCUUU